(2-16-95)

MRID No. 428990-04

DATA EVALUATION RECORD

1. **CHEMICAL:** Oxine Copper. Shaughnessey Number: 024002.

2. **TEST MATERIAL:** 1) Quinolate 98; oxine copper or copper 8-quinolinolate; Batch No. 52390; 100% active ingredient; a green powder. 2) ^{14}C-oxine copper; Lot No. 041H9267; specific activity of 22.5 mCi/mmol; 98% active ingredient.

3. **STUDY TYPE:** 72-3. Mollusc 48-hour Embryo-Larval Study. Species Tested: Eastern Oyster (*Crassostrea virginica*).

4. **CITATION:** Ward, G.S. and J. Davis. 1993. Oxine Copper (Copper 8-Quinolinolate): Acute Toxicity to Embryos and Larvae of the Eastern Oyster, *Crassostrea virginica*, Under Static Test Conditions. Laboratory Project ID No. J9006014i. Study performed by Toxikon Environmental Sciences, Jupiter, FL. Submitted by LA QUINOLEINE et ses dérivés, S.A., France. EPA MRID No. 428990-04.

5. **REVIEWED BY:**

Rosemary Graham Mora, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Rosemary Graham Mora
Date: 15 Dec, 1993

Joseph Sylvester 2/16/95

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 12/15/93

James J. Goodyear, Ph.D.
Project Officer, EEB/EFED
USEPA

Signature: Henry T. Craven
Date: 2/16/95

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an acute toxicity study using mollusc embryos and larvae. Based on percentage reduction in normal development of larvae and mean measured concentrations, the 48-hour EC_{50} was 36.3 µg/l which classifies oxine copper as very highly toxic to *Crassostrea virginica*. The NOEC was 11.1 µg/l mean measured concentration.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Embryos of the Eastern oyster (*Crassostrea virginica*) were obtained by inducing oysters to spawn. Adult oysters were obtained on the day of test initiation from a commercial supplier in Fort Pierce, FL. The adult oysters were placed in a glass chamber containing 3-4 l of dilution water and were induced to spawn by raising the water temperature to 30°C. At the onset of spawning, individual oysters were placed in smaller glass dishes containing 300 ml of filtered saltwater. Following spawning, 3 ml of live sperm suspension from a sacrificed male were added to fertilize the eggs of a female. Microscopic confirmation estimated 90% fertilization.

B. **Test System:** The study was conducted in 1.5-l glass dishes, each containing 1 l of test solution. Four replicates of the control and three replicates of each test concentration and the solvent control were prepared.

The test temperature was maintained at 20 ±1°C by controlling the room temperature. A photoperiod of 16 hours of light at a light intensity of 667-1917 lux was provided.

The dilution water was natural saltwater collected from a shallow saltwater well. The seawater was filtered (5 and 0.45 µm), sterilized, and adjusted to a salinity of 30 parts per thousand (ppt) with deionized water.

A mixed stock solution (1032 mg/l) was prepared by adding appropriate amounts of unlabelled and radiolabelled test material to a 100-ml flask with 8 drops of hydrochloric acid and bringing it to volume with dimethylformamide (DMF). A primary stock solution was prepared by diluting 4.84 ml of the mixed stock solution with DMF to a total volume of 10 ml. This primary stock was serially diluted to prepare five additional stock solutions. A total of 0.3 ml of each stock was added to 3 l of dilution water to prepare the test solutions.

C. **Dosage:** Forty-eight-hour, static test. Six nominal concentrations (3.89, 6.5, 10.8, 18, 30, and 50 µg/l)

were used in this study. In addition, a dilution water control and a solvent control were included. The solvent concentration in the solvent control and all test solutions was 100 µl DMF/l.

D. **Design:** At the start of the test, 1.1 ml of an inoculum of embryo suspension with a density of 18,200 embryos/ml was added to each vessel. The test chambers were positioned on a shelf in a temperature-controlled chamber. The four dilution water control replicates were sampled for determination of the initial embryo loading.

After 48 hours, each vessel was mixed with a perforated plunger, and 10 ml of solution removed for enumeration of normal and abnormal oyster larvae using a Sedgewick-Rafter counting cell.

The temperature was measured daily in one replicate of the dilution water control, and continuously in the temperature-controlled chamber. The salinity of the dilution water control was determined at test initiation. At the start of the test, the pH and dissolved oxygen concentration (DO) were measured in the prepared test solutions prior to separating replicates. At test termination, the pH and DO in one replicate of each solution were measured.

Each test solution was sampled at test initiation and termination for determination of the test substance concentrations using liquid scintillation counting.

E. **Statistics:** Results of the toxicity test were used to calculate the percentage reduction of normal oyster larvae of each test concentration when compared to the pooled control data. The median effective concentration (EC_{50}) and 95% confidence limits were determined using a computer program (Stephan, 1977).

Statistical difference in the number of normal larvae between the pooled control and the exposure concentrations were determined using analysis of variance (ANOVA) and Dunnett's comparison procedure.

12. **REPORTED RESULTS:** Mean measured concentrations were 3.94, 6.51, 11.1, 18.4, 29.7, and 50.3 µg/l which represent 99-102% of nominal concentrations (Table 1, attached).

Based on the number of normal larvae and mean measured concentrations, the 48-hour EC_{50} was 37.8 µg/l with a 95% confidence interval of 29.7-50.3 µg/l, and the no-observed effect concentration (NOEC) was 11.1 µg/l (Table 2, attached).

During the test, the test solutions had a DO of 7.0-7.5 mg/l and a pH of 8.5-8.9. The solution temperature ranged from 19.1-20.1°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The author made no conclusions in the report.

A GLP compliance statement and a quality assurance statement were included in the report indicating that the study was conducted in accordance with Good Laboratory Practice regulations under FIFRA.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedures were generally in accordance with the SEP, but deviated as follows:

According to the author, the initial embryo loading of the four dilution water control replicates was measured at test initiation, but the results were not reported. The reviewer's calculation from the reported inoculum was approximately 20 oysters/l.

The authors did not report percentage of mortality or the EC_{50} based on mortality of oyster embryos and larvae.

The results of the salinity measurements were not reported.

The SEP states that embryos should be tested within one hour of spawning and after fertilization. The author did not report the post-fertilization age of the test organisms.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal computer program to calculate the 48-hour EC_{50} (95% confidence interval) for the reduction in normal development of oyster larvae (printout, attached). The EC_{50} (95% confidence interval) was 36.3 (33.5-39.9) µg/l which is more conservative than the results presented by the author. The NOEC was determined using William's test and was the same as that of the author.

C. **Discussion/Results:** Based on percentage reduction in normal development of larvae and mean measured concentrations, the 48-hour EC_{50} was 36.3 µg/l which classifies oxine copper as very highly toxic to *Crassostrea virginica*. The NOEC was 11.1 µg/l mean measured concentration.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** N/A.

(3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes; 29 November 1993.

Table 1. Measured Concentrations of Oxine Copper During a 48-Hour Exposure of Embryos and Larvae of the Eastern Oyster, *Crassostrea virginica*, Under Static Conditions

Nominal Concentration (µg/L)	Measured Concentration (µg/L) 0 Hours			48 Hours			Mean (±SD)	Percent of Nominal
	Rep A	Rep B	Rep C	Rep A	Rep B	Rep C		
Control	<0.28	<0.28	<0.28	<0.28	<0.28	<0.28	<0.28 (0.00)	---
Solvent Control	<0.28	<0.28	<0.28	<0.28	<0.28	<0.28	<0.28 (0.00)	---
3.89	3.73	3.84	3.93	4.14	3.99	4.02	3.94 (0.14)	101
6.5	6.33	6.21	6.23	6.69	6.88	6.72	6.51 (0.29)	100
10.8	10.7	10.8	11.0	11.3	11.3	11.3	11.1 (0.31)	102
18.0	17.7	17.9	17.8	19.1	18.8	18.8	18.4 (0.60)	102
30.0	28.9	28.6	29.4	30.7	30.3	30.5	29.7 (0.89)	99
50.0	48.4	48.3	48.2	52.9	52.6	51.5	50.3 (2.23)	101
MATRIX SPIKE QC DATA								
MS		3.76(QC-1) 48.7 (QC-2)			3.96(QC-3) 48.5 (QC-4)		3.86 48.6	93 94

SD = Standard Deviation.
MS = Matrix spike. The matrix spike consisted of test substance in dilution water. The spike concentration was 4.13 µg/L for QC-1 and QC-3 while the concentration was 51.60 µg/L for QC-2 and QC-4.

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Oxine Copper: Number of Normally Devloped Oyster Larvae
File: b:42899004.oys Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.407	5.082	8.022	5.082	1.407
OBSERVED	0	8	6	7	0

Calculated Chi-Square goodness of fit test statistic = 5.7230
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Oxine Copper: Number of Normally Devloped Oyster Larvae
File: b:42899004.oys Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 26.833

W = 0.979

Critical W (P = 0.05) (n = 21) = 0.908
Critical W (P = 0.01) (n = 21) = 0.873

Data PASS normality test at P=0.01 level. Continue analysis.

Oxine Copper: Number of Normally Devloped Oyster Larvae
File: b:42899004.oys Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 12.25
Closest, conservative, Table H statistic = 1705.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 7, df (# reps-1) = 2
Actual values ==> R (# groups) = 7, df (# avg reps-1) = 2.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

Oxine Copper: Number of Normally Devloped Oyster Larvae
File: b:42899004.oys Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 3.86
Table Chi-square value = 16.81 (alpha = 0.01)
Table Chi-square value = 12.59 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.00
Used for Chi-square table value ==> df (#groups-1) = 6

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

TITLE: Oxine Copper: Number of Normally Devloped Oyster Larvae
FILE: b:42899004.oys
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Solvent Control	1	16.5000	16.5000
1	Solvent Control	2	18.5000	18.5000
1	Solvent Control	3	17.0000	17.0000
2	3.94	1	15.0000	15.0000
2	3.94	2	14.5000	14.5000
2	3.94	3	16.0000	16.0000
3	6.51	1	16.0000	16.0000
3	6.51	2	17.5000	17.5000
3	6.51	3	15.0000	15.0000
4	11.1	1	16.0000	16.0000
4	11.1	2	17.0000	17.0000
4	11.1	3	16.0000	16.0000
5	18.4	1	12.0000	12.0000
5	18.4	2	16.0000	16.0000
5	18.4	3	14.5000	14.5000
6	29.7	1	15.0000	15.0000
6	29.7	2	14.5000	14.5000
6	29.7	3	12.5000	12.5000
7	50.3	1	4.0000	4.0000
7	50.3	2	0.0000	0.0000
7	50.3	3	2.0000	2.0000

Oxine Copper: Number of Normally Devloped Oyster Larvae
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Solvent Control	3	17.333	17.333	17.333
2	3.94	3	15.167	15.167	15.889
3	6.51	3	16.167	16.167	15.889
4	11.1	3	16.333	16.333	15.889
5	18.4	3	14.167	14.167	14.167
6	29.7	3	14.000	14.000	14.000
7	50.3	3	2.000	2.000	2.000

.ne Copper: Number of Normally Devloped Oyster Larvae
File: b:42899004.oys Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Solvent Control	17.333				
3.94	15.889	1.278		1.76	k= 1, v=14
6.51	15.889	1.278		1.85	k= 2, v=14
11.1	15.889	1.278		1.88	k= 3, v=14
18.4	14.167	2.801	*	1.89	k= 4, v=14
29.7	14.000	2.949	*	1.90	k= 5, v=14
50.3	2.000	13.565	*	1.91	k= 6, v=14

1.384
Note: df used for table values are approximate when v > 20.

Rosemary Graham Mora Oxine Cu Oyster

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
50.3	100	88	88	0
29.7	100	19	19	0
18.4	100	18	18	0
11.1	100	6	6	0
6.51	100	6	6	0
3.94	100	12	12	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 37.38728

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	3.230735E-02	36.31254	33.49868	39.86267

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	1.744854	25.53114	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 2.019905

95 PERCENT CONFIDENCE LIMITS =-.6482465 AND 4.688056

LC50 = 37.33397

95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 8.777314

95 PERCENT CONFIDENCE LIMITS = 0 AND 24.32801

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